Postpartum administration of Citalopram reverses gestational stress-induced depressive-like behavior and structural modifications in the reward pathway

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Abstract

Postpartum depression (PPD) is a common complication following childbirth experienced by approximately 20% of new mothers. We have previously shown that chronic gestational stress, a risk factor for PPD, induces depressive-like behavior in postpartum rats and impairs maternal care, a rewarding, motivated behavior. These behavioral consequences of gestational stress are accompanied by structural changes on neurons in the nucleus accumbens (NAc), a key brain region in the reward pathway which is involved in maternal care and which has been implicated in PPD. Here, we extended our previous work in two experiments. First, we examined the effects of gestational stress on other reward-related behaviors known to be altered in mothers with postpartum depression including anhedonia (as assessed with the sucrose preference test) and maternal motivation (as assessed with the conditioned place preference paradigm). Second, because mothers diagnosed with PPD are often prescribed selective serotonin reuptake inhibitors (SSRI) antidepressants to ameliorate mood and other deleterious effects of PPD, we investigated the extent to which the SSRI Citalopram could reverse stress-induced depressive-like behavior and morphological changes in the NAc. Our results show that along with increased depressive-like behavior, postpartum females exposed to chronic stress during pregnancy (from GD7-GD20) exhibited anhedonia, deficits in maternal motivation as well as structural modifications in the NAc. We also found that postpartum administration of Citalopram was able to reverse the depressive-like behavior and the structural modifications in the NAc of gestationally stressed mothers. Overall, our results demonstrate that gestational stress induces numerous behavioral symptoms found in depressed mothers and that depressive-like behavior in gestationally stressed mothers is responsive to antidepressant treatment. In doing so, these results expand the validity

of our gestational stress model and suggest that structural plasticity in the NAc pathway may play a critical role in mediating depressive-like behavior in PPD.

Introduction

Having a baby can be one of the happiest and most monumental events in a woman's life. However, alterations in mood during the postpartum period are reported by approximately 40% of all new mothers with up to one in every five mothers developing the full phenotype of major depression known as postpartum depression (PPD; Gress-Smith et al., 2012; O'Hara, 2009; O'Hara and Wisner, 2014). In addition to classical depressive symptoms such as anhedonia, lack of motivation, and hopelessness, mother-infant interactions are also adversely affected and are often characterized by low levels of physical contact, attachment issues and infrequent feeding behaviors (Lovejoy et al., 2000). As a result, the well-being and development of the offspring can be compromised (Grace et al., 2003; Gress-Smith et al., 2012; Letourneau et al., 2012; Verbeek et al., 2012). Indeed, children of mothers suffering from postpartum depression have a higher risk of developing mood disorders, attention and cognitive deficits, and poor social capabilities (Field, 2010). Thus, understanding the neurobiological mechanisms underlying postpartum depression (PPD) is critical for the health of both the mother and child.

Epidemiological studies have revealed that exposure to stress during pregnancy is a major factor which enhances vulnerability to PPD (O'Hara and Wisner, 2014). Similarly, in rodents, chronic gestational stress or exposure to stress hormones during pregnancy increases postpartum depressive-like behavior (Smith et al, 2004, O'Mahoney et al.,2006; Green et al., 2009; Haim et al., 2014; Leuner et al., 2014;; Perani and Slattery, 2014) and impairs maternal care (Leuner et al., 2014; Haim et al., 2014). Pups are highly rewarding for mother rats and maternal care is considered a highly motivated behavior (Numan and Insel, 2003; Lee et al, 1999) which suggests that depressed mothers exposed to gestational stress experience reward-related deficits. To explore the possibility that other reward-related behaviors are adversely affected by gestational

stress, we examined the extent to which gestational stress induces anhedonia and impaired maternal motivation during the postpartum period.

Motivated behaviors including maternal behavior are mediated by increased activity of the mesolimbic pathway that sends dopaminergic projections from the ventral tegmental area (VTA) to several forebrain regions including the nucleus accumbens (NAc), the center for reward and motivation processing (Russo & Nestler, 2013). Recent research also highlights the role of this pathway in mood disorders including PPD (Nestler & Carlezon., 2006; Russo and Nestler 2013). For example, fMRI studies in mothers with PPD have shown that exposure to rewarding stimuli or infant cries produces less activation in the ventral striatum as compared to healthy mothers (Moses-Kolko et al., 2011; Moses-Kolko et at., 2012). In accordance with these human studies, data from our lab have demonstrated a reduction in neuronal complexity and dendritic spine density in the NAc of postpartum rats following chronic gestational stress (Haim et al., 2014). Together, these data suggest that aberrations in reward pathway may contribute to the symptoms of PPD.

Selective serotonin reuptake inhibitors (SSRI) are antidepressants commonly used to treat depressive-like symptoms, including PPD (Berle and Spigset, 2011; Logsdon et al., 2011). However, not much is known about the effects these medications have on the maternal brain. Therefore, we also examined whether a frequently used SSRI, Citalopram (Celexa©) (Rampono et al., 2006l; Misri et al., 2012), could mitigate depressive-like symptoms in postpartum rats exposed to chronic gestational stress and the extent to which Citalopram could reverse the structural changes in the NAc of gestationally stressed mothers.

Materials and Methods

Animals. Timed pregnant female Sprague-Dawley rats (Taconic, Albany, USA) arrived at our facility on gestation day 4 (GD4) and were individually housed in clear Plexiglas cages with unlimited access to food and water. Rats were kept in a temperature and humidity controlled environment maintained on 12h/12h light-dark cycle (lights on at 6AM). The day of pup delivery was designated as postpartum day 0 (PD0). On PD1, litters were culled to 8-10 pups (4-5 males, 4-5 females) in a randomized manner to minimize the possibility that the effects observed in the stressed mothers are driven by the characteristics of their pups. For Experiment 2, rats were weighed daily from GD7-GD20 and both mothers and litters were weighed daily throughout the postpartum period. All experiments were performed in compliance with The Ohio State University Institutional Animal Care and Use Committee and the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experiment 1: Effects of gestational stress on anhedonia and maternal motivation

Stress procedure. Pregnant rats were randomly assigned to the stressed group or served as unstressed controls. Unstressed controls were handled daily for 5 min. Stressed animals underwent a chronic variable stress protocol (Hill et al, 2012) that includes randomized daily exposure to two of the following from GD7-GD20: restraint stress 1 x 45 min and 1x 2 hours, social defeat for 1 x 30 min, overnight damp bedding, foot shock, overnight food deprivation, and 12 hour overcrowding. Multiple stress sessions within the same day were always separated by at least 1 h. Randomized variable restraint stress was used in this study to reduce stress predictability and potential habituation to the stress paradigm. Separate groups of stressed and unstressed mothers were subjected to the sucrose preference test for anhedonia (No stress, n=9;

Stress, n=9) or the conditioned place preference paradigm to assess maternal motivation (No stress, n=6; Stress, n=5).

Sucrose Preference Test. Anhedonia was assessed using the sucrose preference test (SPT). From PD3-5, home cages were disconnected from the watering system and postpartum females were habituated to drinking from two water bottles places in their cages, both containing their usual drinking water. Water bottles were removed daily at 3pm and returned to cages at 6pm. This brief water deprivation was used to increase the motivation for fluid consumption. Following water deprivation on PD6 and PD7, one of the bottles was replaced with a bottle containing 2% sucrose. The positions of the two bottles was counterbalanced and switched after one day to prevent preferential drinking based on location in the cage. The consumption of water and sucrose was monitored for the first hour into the active phase (6pm-7pm). Afterwards the sucrose bottle was removed and replaced with a water bottle. Sucrose preference was calculated by averaging the amount of sucrose consumed on PD6 and PD7, divided by the average daily water consumption across the three days of habituation.

Conditioned place preference paradigm. The conditioned place preference paradigm (CPP) was used to assess maternal motivation. For CPP, the apparatus consisted of two visually distinct (one black, one white) equal sized chambers (28 x 28 x 20 cm) made of Plexiglas. The chambers were connected by a manually operated guillotine door. The test consisted of three phases that occurred over 6 consecutive days: a pre-conditioning baseline session (pre-test), a conditioning phase and a post-conditioning test phase. The pre-test occurred on PD1. Mother rats were placed in the apparatus and allowed to explore both chambers for 15 min to identify pre-conditioning chamber preferences as well as to habituate the rats to the apparatus. Importantly, all rats significantly preferred the dark chamber over the white chamber coinciding with rodent's natural

tendency to prefer dark environments. As such, pups were paired with the white chamber to avoid the natural tendency to spend more time in the dark chamber. Conditioning took place from PD2-5 during which the divider separating the chambers was lowered. Rats underwent one conditioning session per day in which 3 pups from the litter for which they were caring in their home cages and which were age-matched to the postpartum day of the mother were paired with the white chamber or 3 pup sized objects (3 cm long plastic tubing, 1 cm diameter) were paired with the dark chamber (Wansaw et al., 2008). For each conditioning session, rats were placed in one of the side chambers with the stimulus for 2 hr. Conditioning was done in an alternating sequence such that on one day pups were conditioned to the white chamber and on the following day pup sized objects were conditioned to the dark chamber. The order of the presentation of the stimuli was counterbalanced; therefore, half of the mothers received pups on the first conditioning day, the other half received tubing. On the day of testing (PD6), mothers were separated from pups for 15 min and then placed in the apparatus and allowed to freely explore with no stimuli present for 2 hr. All phases of the CPP test were digitally recorded. Only the first 15 min of behavior were analyzed due to very low levels of exploration during the remainder of the test period. To calculate the preference ratio, the time spent in the white chamber during the first 15 min of the pre-test on PD1 was divided by the time spent in the white chamber during the first 15 min of the PD6 test.

Experiment 2: Effects of postpartum antidepressant administration on gestational stress-induced depressive-like behavioral and structural plasticity in the NAc

Stress procedure. Pregnant rats were randomly assigned to the stressed group or served as unstressed controls. Unstressed controls were handled daily for 5 min. Stressed animals

underwent a chronic restraint stress protocol randomly consisting of one of the following per day from GD7-GD20: restraint stress 2 X 30 min, 3 X 45 min, 1 X 1 h and 1 X 2 h. Multiple stress sessions within the same day were always separated by at least 1 h. Randomized variable restraint stress was used in this study to reduce stress predictability and potential habituation to the stress paradigm.

Minipump implantation and antidepressant administration. On PD1, postpartum females were randomly assigned to receive Citalopram hydrobromide (generous gift from H. Lundbeck, Copenhagen, DK; 10mg/kg/d in saline) or saline vehicle resulting in a total of 4 groups: (1) No stress-Saline (n=8), (2) No stress-Citalopram (n=8), (3) Stress-Saline (n=7), (4) Stress-Citalopram (n=7). Citalopram treatment was administered for 21 d via osmotic minipumps (2ML4, Alzet, Cupertino, CA) which were preloaded with 2 ml of Saline or Citalopram and subcutaneously implanted between the scapulae under Isoflurane anesthesia. At the end the study, fluids from each minipump were aspirated to verify saline/drug delivery. During the surgery, litters remained in their home cages which were kept warm on a heating pad. Following implantation, mothers were placed back in their home cages and provided with ibuprofen via drinking water for 7 d.

Forced swim test. The forced swim test (FST) was used to assess behavioral despair. Plexiglas cylinders (diameter: 30.5 cm, height: 49 cm) were filled to a depth of 30 cm with 25 ± 0.5°C water. On PD21, postpartum females were individually placed into the FST cylinders for 10 min, towel-dried and returned to their home cage. 24 h later (PD22), rats were returned to the same apparatus for 5 min and the session was digitally recorded. The percentage of time spent immobile [(time spent floating in the water only making movements necessary to maintain the

head above water/total test time) x 100] was later measured blind by a trained observer using BEST analysis software (Education Consulting Inc., Hobe Sound, FL).

Golgi staining. 24 h following the FST, postpartum females (PD23) were deeply anesthetized, rapidly decapitated and had brains removed for Golgi impregnation using the FD Rapid Golgi Stain kit (FD Neurotechnologies; Ellicot City, MD). Briefly, small blocks of tissue containing the NAc were placed in plastic scintillation vials filled with 10 ml of a potassium dichromate, mercuric chloride and potassium chromate solution (Solution A+B). Following two weeks of incubation in the dark at room temperature, brains were transferred to solution C and stored in the dark at 4°C for 2 d. Next, coronal sections (150 μm) were cut on a Vibratome, mounted onto gelatin-coated slides and dried at room temperature in the dark. Slides were then rinsed, developed in solutions D + E for 10 min, dehydrated, cleared with xylene and coverslipped with Permount.

Microscopic analyses. MSNs (1.7mm and 1mm anterior to Bregma; Paxinos and Watson, 1998) in the shell sub-region of the NAc were analyzed. We focused on this region because our prior work has shown that MSNs in the shell,but not core, are sensitive to gestational stress (Haim et al., 2014). Only neurons within this region that were fully impregnated, not obscured by neighboring neurons, and had no obviously truncated dendrites were chosen for analysis. For each animal, five randomly chosen, representative neurons were completely traced. From these traced neurons, total dendritic length and number of branch points (every point of bifurcation along dendritic branches) were measured. Further, on these neurons, dendritic spines were counted at 100X on five dendritic segments 20 μm in length located at least 50 μm away from the cell body. All analyses were performed blind to experimental conditions.

Spine density was calculated by dividing the number of spines on a segment by the segment length and expressed as the numbers of dendritic spines per 10 μm. The numbers of spines on five segments of a cell were averaged for a cell mean, and the five cells from each animal were then averaged for an animal mean. For dendritic length and branching, values for each of the five cells per animal were averaged to obtain an animal mean.

Statistical analyses. Group data are reported as the mean ± SEM. Behavior from SPT and CPP were analyzed using two-tailed t-tests. All body weight and litter weight parameters as well as percent immobility in the FST were analyzed using two-way ANOVA with drug (saline vs. Citalopram) and stress condition (stress vs. no stress) as independent variables. Neuronal morphology (*i.e.* total dendritic length, number of branch points and spine density) data was analyzed using two-way ANOVA with drug and stress condition as independent variables. Bonferroni post-hoc tests were applied when necessary. All analyses were conducted using GraphPad Prism 5.0 software (La Jolla, CA) with significance set at $p \leq 0.05$.

Results

Experiment 1: Effects of gestational stress on reward-related behaviors

Gestational stress had a negative impact on the two reward-related behaviors measured-maternal motivation and anhedonia. As shown in Figure 1, maternal motivation on the CPP test was impaired by gestational stress [$t(9) = 3.24$, $p < 0.05$]. Unstressed mothers developed a preference for the pup-associated chamber as indicated by a preference ratio of >1. In contrast stressed mothers failed to develop a preference for the pup associated chamber.

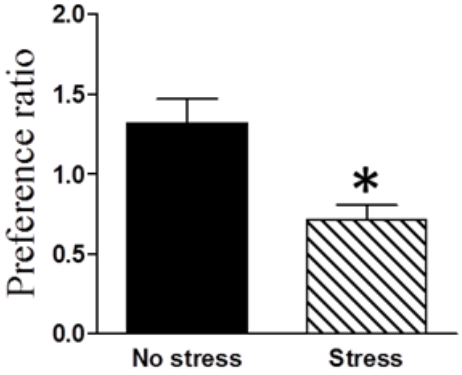

Figure 1. Gestational stress impairs maternal motivation. Unstressed mothers developed a place preference for the pup-associated chamber (preference ratio >1) indicating an increased maternal motivation during the postpartum period. In contrast, mothers that underwent gestational stress failed to develop a conditioned place preference indicative of reduced maternal motivation. Bars represent mean ± SEM; * $p<0.05$

Gestational stress also induced anhedonia in postpartum females. As shown in Figure 2, gestational stress reduced sucrose preference on the SPT [$t(16)=2.438$, $p<0.05$].

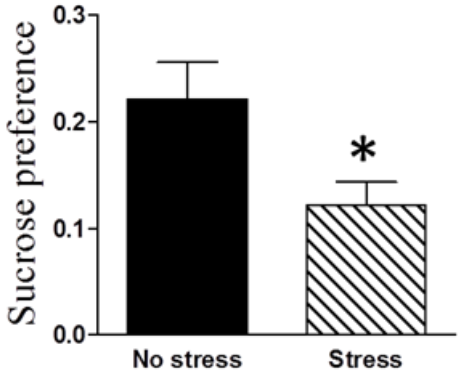

Figure 2. Gestational stress induces anhedonia in postpartum females. As compared to unstressed mothers, sucrose preference during the SPT was reduced mothers exposed to gestational stress. Bars represent mean ± SEM; * $p<0.05$

Experiment 2: Effects of gestational stress and Citalopram, on stress-induced depressive-like behavior and structural plasticity in the NAc

Weight gain and litter characteristics. As seen in Table 1, a significant main effect of stress [$F(1,30) = 4.13$, $p = 0.05$] on percent body weight gain during pregnancy was found such that pregnant females who were stressed gained less weight. There was no main effect of drug and no stress x drug interaction on gestational weight gain (p's > 0.05). For postpartum weight gain, there were no main effects of gestational stress or drug and no stress x drug interaction (p's > 0.05).

With regard to the pups, there was no main effect of stress or drug (p's > 0.05) but a significant stress x drug interaction [$F(1,30) = 4.24$ $p < 0.05$] for percent litter weight gain. Post-hoc analysis, however, showed no significant differences between the groups. No significant main effects or interactions were found for litter size (No stress-Saline: 10.25 ± 4.80 pups; No-stress-Citalopram: 9.87± 3.13 pups; Stress-Saline: 12.00 ± 3.08 pups; Stress-Citalopram: 10.55 ± 5.15 pups, p's > 0.05) or litter weight on PD1 (p's > 0.05).

Group	% Gestational weight gain	% Postpartum weight gain	Litter weight on PD1 (g)	% Litter weight gain
No stress Saline	34.37 ± 2.76	18.08 ± 1.75	70.12 ± 10.89	507.08 ± 28.57
No stress Citalopram	36.37 ± 2.76	21.41 ± 1.98	72.62 ± 11.78	551.71 ± 30.78
Stress Saline	30.56 ± 2.95*	21.36 ± 1.15	77.14 ± 9.68	532.63 ± 35.47
Stress Citalopram	27.03 ± 2.97*	19.60 ± 1.89	68.57 ± 10.45	472.95 ± 32.49

Table 1. Effects of gestational stress and chronic postpartum administration of Citalopram on percent weight gain during pregnancy and the postpartum period. Also shown is litter weight on PD1 and percent litter weight gain. *$p < 0.05$, main effect of stress.

Depressive-like behavior. A significant main effect of both stress [$F(1,26) = 6.61$, $p < 0.05$] and drug [$F(1,26) = 6.75$, $p < 0.05$] as well as a significant stress x drug interaction [$F(1,26) = 4.24$, $p < 0.05$] was found for percent immobility in the FST (Figure 3). Post-hoc analysis showed that the Stress-Saline group displayed more immobility in the FST as compared to all other groups ($p < 0.5$) which did not differ from one another (p's > 0.05).

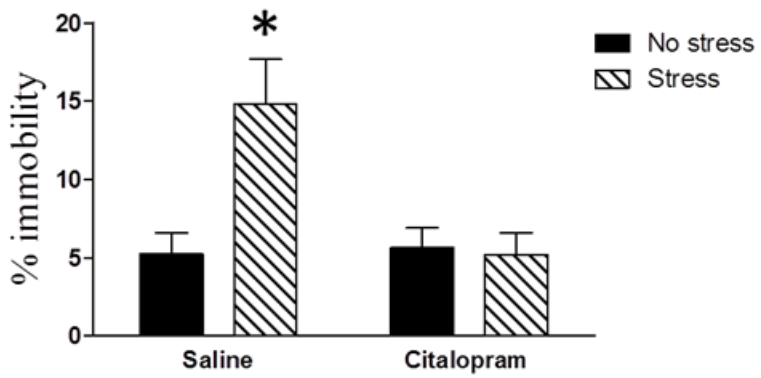

Figure 3. Chronic Citalopram administration reverses gestational stress-induced depressive-like behavior during the postpartum period. Gestational stress significantly increased percent immobility in the forced swim test indicative of depressive-like behavior. Postpartum females treated with Citalopram did not exhibit increased immobility following gestational stress. Bars represent mean ± SEM. # $p < 0.05$, Stress-Saline vs. all other groups.

Structural plasticity. For total dendritic length of NAc shell MSNs (Figure 5a), there was no main effect of stress [$F(1,26) = 2.76$, $p > 0.05$] but a significant main effect of drug [$F(1,26) = 13.09$, $p<0.01$] and a significant stress x drug interaction [$F(1,26) = 5.95$, $p < 0.05$]. Post hoc analysis revealed that total dendritic length was significantly reduced in the Stress-Saline group in comparison with all other groups (p's < 0.05) which did not differ (p's > 0.05). For number of branch points on NAc shell MSNs (Figure 5b), there were significant main effects of stress [$F(1,26) = 10.04$, $p<0.01$] and drug [$F(1,26) = 11.57$, $p<0.01$] as well as a significant interaction

between the two factors [$F(1,26) = 4.44$, $p<0.05$]. Post-hoc analysis revealed that dendritic branching was reduced in the Stress-Saline group in comparison with all other groups (p's < 0.05) which did not differ from one another (p's > 0.05). Lastly, for dendritic spine density on NAc MSNs (Figure 5c), significant main effects of stress [$F(1,26) = 12.35$, $p<0.01$] and drug [$F(1,26) = 7.21$, $p<0.05$] as well as a significant stress x drug interaction [$F(1,26) = 4.44$, $p<0.05$] were found. Once again, post-hoc analysis showed that spine density was reduced in the Stress-Saline group in comparison with all other groups (p's < 0.05) which did not differ (p's > 0.05).

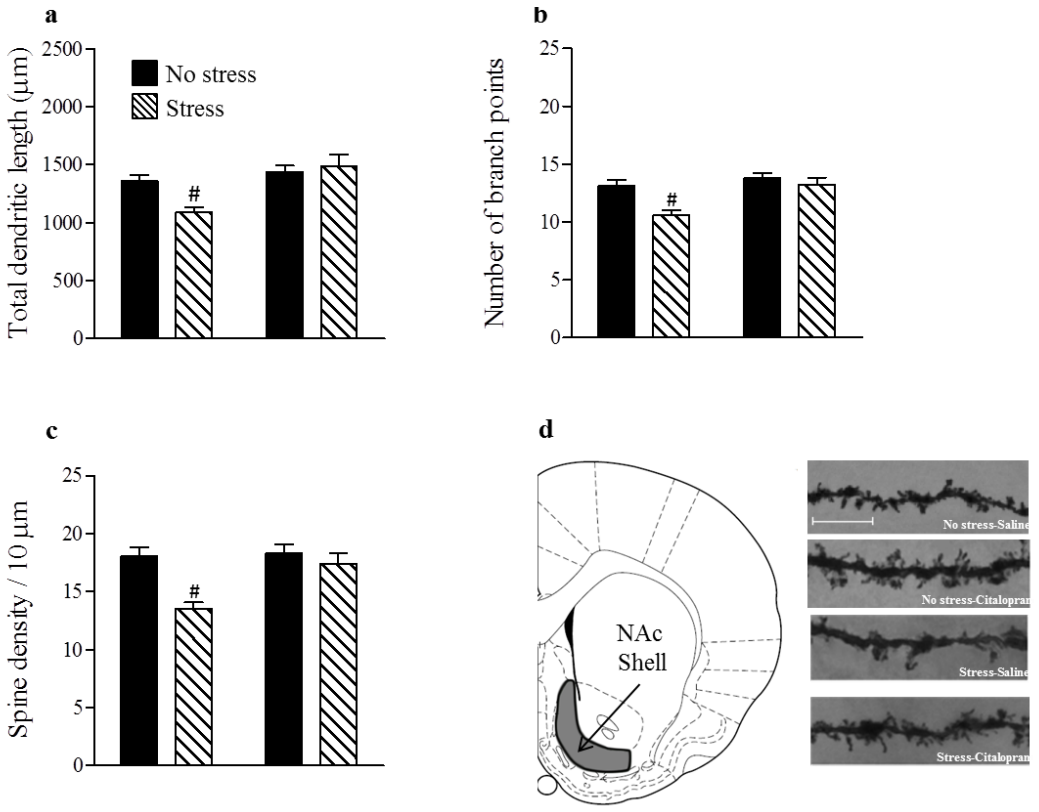

Figure 4. Chronic Citalopram administration reverses the effects of gestational stress on structural plasticity within the postpartum NAc shell. Gestational stress significantly reduced the total dendritic length (a), number of branch points (b), and dendritic spine density (c) on MSNs in the NAc shell (d). For all three morphological measurements, chronic postpartum administration of Citalopram reversed the effects of gestational stress. Bars represent mean ± SEM. # $p < 0.05$, Stress-Saline vs. all other groups.

Discussion

Here we show that chronic gestational stress alters postpartum behavior by inducing depressive-like symptoms, anhedonia and impairments in maternal motivation. We also demonstrate that postpartum administration of Citalopram, an SSRI commonly prescribed to mothers diagnosed with PPD, mitigated gestational stress-induced depressive-like behavior and reversed structural modifications within the NAc shell during the postpartum period of gestationally stressed mothers. Overall, these data suggests that gestational stress is a translationlly relevant model to study the underlying neurological mechanisms of PPD which our results suggest may involve compromised structural plasticity in the NAC.

Gestational stress induces anhedonia and impairs maternal motivation

In a previous study (Leuner et al., 2014), we found that increased depressive-like behavior in mothers exposed to chronic gestational stress is accompanied by impaired maternal care. Since pups are known to be highly rewarding to postpartum females (Lee et al., 1999; Numan and Insel, 2003), we evaluated the possibility that other reward-related behaviors would also be adversely affected by gestational stress. To do so we used the SPT to assess anhedonia and the CPP test to assess maternal motivation. On the SPT, we found that gestationally stressed mothers were anhedonic and consumed less sucrose than unstressed mothers. It is important to point out that the transition to motherhood itself is accompanied by an endogenous reduction of sucrose intake (Green et al, 2009). Since we found a significant reduction of sucrose intake in our stressed animals in comparison to controls, our data therefore suggests that this phenomenon is exacerbated by gestational stress.

In addition to anhedonia, gestationally stressed mothers also exhibited impaired maternal motivation on the CPP test. CPP has been used extensively to evaluate the motivational properties of pups (Pereira and Morrell, 2010). For example, it has been shown that early postpartum female rats prefer to spend time in a chamber previously associated with pups than in the chamber previously associated with cocaine indicating that pups have high incentive value for mothers (Mattson et al., 2001). Here we show that gestationally stressed mothers failed to develop a CPP place preference for pups. Based on these findings, we hypothesize that gestational stress may interfere with the ability of mothers to experience pups as rewarding and as a result they may be less motivated to engage in maternal care.

Postpartum administration of Citalopram reverses gestational stress-induced depressive-like behavior and structural modifications in the reward pathway

Selective serotonin reuptake inhibitors (SSRI) are antidepressants commonly used to treat depressive-like symptoms, including PPD (Misri et al., 2012). However, little is known about the effects these medications have on the maternal brain. Therefore, we also examined whether a frequently used SSRI, Citalopram, could mitigate depressive-like symptoms in postpartum rats exposed to chronic gestational stress and the extent to which Citalopram could reverse the structural changes in the NAc of gestationally stressed mothers.

In this study, we assessed body weight and litter weight parameters of the mothers and pups and we found that regardless of antidepressant treatment, gestational stress decreased pregnancy weight gain. Thus, these data validate our stress paradigm and are in line with other studies which have demonstrated a decrease in pregnancy weight gain following gestational stress (Baker et al., 2008; Haim et al., 2014; Hillerer et al., 2011; Leuner et al., 2014). Moreover,

they support research in humans showing a positive link between pregnancy stress and insufficient pregnancy weight gain (Brawarsky et al., 2005; Picone et al., 1982). In contrast with other studies (Hillerer et al., 2011; Leuner et al., 2014) however, we found no effect of gestational stress on litters' or mothers' weight gain during the postpartum period, a discrepancy likely attributable to different stress protocols or the randomized pup culling procedure used here which controls for the possible prenatal stress exposure effects.

Although previous work has shown that chronic gestational stress increases depressive-like behavior during the postpartum period (Haim et al., 2014; Hillerer et al., 2011; Leuner et al., 2014; O'Mahony et al., 2006; Smith et al., 2004), our results demonstrate for the first time that chronic postpartum administration of the antidepressant Citalopram is effective in ameliorating postpartum depressive-like behavior in mothers exposed to gestational stress. Specifically, gestationally-stressed mothers treated with Citalopram during the postpartum period spent significantly less time immobile in the FST when compared to gestationally-stressed mothers that received saline and did not differ from mothers who were unstressed. Although, a similar reversal of stress-induced immobility following chronic Citalopram administration has been observed in male rats (Chen et al., 2012), the effects of chronic SSRI administration on depressive-like behavior in gestationally stressed mothers has only been examined in one other study which found no effect of gestational stress or antidepressant treatment on immobility in the FST (Pawluski et al., 2012). However, it is important to consider that a different SSRI (fluoxetine) and stress protocol were used and mothers were tested several days after weaning. Nonetheless, research in human mothers diagnosed with PPD indicates that SSRIs such as Citalopram and fluoxetine are generally effective in ameliorating mood (Misri et al., 2012; Berle and Spigset, 2011). Thus, Citalopram's ability to reverse gestational stress-induced depressive-

like behavior in postpartum rats increases the validity of gestational stress as a translational model for PPD.

In addition to ameliorating stress-induced depressive-like behavior, postpartum administration of Citalopram also reversed structural modifications in the NAc of mothers exposed to chronic gestational stress thereby confirming and extending our prior work (Leuner et al., 2014; Haim et al., 2014). These included stress-induced reductions in total dendritic length, number of branch points and spine density on NAc shell MSNs. Although neuroimaging work has previously linked this brain region to PPD (Laurent and Ablow, 2012; Moses-Kolko et al., 2010, 2011; Sacher et al., 2015; Silverman et al., 2011), our data provide evidence that stress-induced neuroplastic changes in the postpartum NAc may contribute to the pathophysiology of PPD and its pharmacologically induced recovery.

The transition into motherhood is accompanied by dramatic behavioral and physiological changes (Rosenblatt, 1980; Rosenblatt et al., 1988). Indeed, multifaceted hormonal and neurochemical alterations occur during the postpartum period (Rosenblatt, 1989; Numan and Insel, 2003). Thus, perhaps it should not be too surprising that the morphological findings reported here in postpartum females are complex and in some cases contrast stress effects, and the antidepressant response to those effects, that have been observed in virgin male and/or female rodents. For example, MSNs in the NAc of male rats or mice exposed to chronic stress display dendritic hypertrophy and increased spine density (Bessa et al., 2013; Christoffel et al., 2011), and these effects are reversed by antidepressant treatment (Bessa et al., 2013). Therefore, even though the fine structure of the NAc is differentially affected by stress in males versus postpartum females, both are responsive to antidepressant treatment.

Chronic stress, which induces depressive-like behavior in rodents, has been shown to reduce central serotonin availability (Boyarskikh et al., 2013; Zhang et al., 2012). Moreover, changes in synaptic availability of serotonin are highly associated with neuronal remodeling (Sun and Schacher, 1998; Vetencourt et al., 2011). Thus, alterations in the serotonergic system following stress may be responsible, at least in part, for stress-induced morphological modifications in mood regulating brain regions. By reinstating synaptic availability of serotonin following chronic stress, Citalopram could thereby ameliorate stress-induced structural changes in the NAc. Such restoration of impaired structural plasticity could in turn be a mechanism by which SSRIs mediate their therapeutic effects as has been previously suggested (Licznerski and Duman, 2013). The presence of presynaptic serotonin transporters (5-HT_{1A}, 5-HT_{B}, and 5-HT_{D}), the action sites of SSRIs, in the NAc (Celada et al., 2013; Selvaraj et al., 2014; Van Bockstaele and Pickel, 1993), further supports this possibility. Another possible mechanism by which gestational stress and Citalopram could affect depressive-like behavior and structural plasticity is through the HPA axis (Nemeroff and Owens, 2004). Chronic restraint stress is known to elicit dysregulation in HPA axis activity (Bratt et al., 2001; Mizoguchi et al., 2008) which is implicated in depression (Pariante and Lightman, 2008; Swaab et al., 2005; Varghese and Brown, 2001) including PPD in both humans (Glynn et al., 2013; Holsen et al., 2013) and rodent models (Brummelte and Galea, 2010). High levels of stress hormones resulting from HPA hyperactivity are known to regulate structural plasticity throughout the brain including in the NAc shell (Garrett and Wellman, 2009; Morales-Medina et al., 2009; Rodrigues et al., 2009) of virgin rats. Thus, reinstatement of normal HPA axis activity along with reductions in levels of stress hormones could underlie Citalopram's positive effect on postpartum mood and structural plasticity noted in this study. Lastly, dopamine is known to be a positive regulator of structural

plasticity in the NAc (Russo and Nestler, 2013) and is also largely implicated in reward-associated behaviors (Meredith et al, 1995) and postpartum depression (Moses-Kolko et al., 2012). Therefore disruptions in dopamine circuitry could also be a mediator of the structural and behavioral changes that occur as a consequence of gestational stress.

Conclusion

The present results demonstrate that gestational stress induced depressive-like behavior, anhedonia, and deficits in maternal care and maternal motivation during the postpartum period. These behavioral changes are accompanied by structural changes within the NAc shell. Importantly, postpartum administration of Citalopram was effective in reversing both depressive-like behavior and structural changes within the NAc shell. Together, these observations provide much needed insight into the effects of stress and antidepressant treatment on the postpartum brain and pave the way for future research using this translational model of PPD.

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